Stochastic models for mechanical transduction
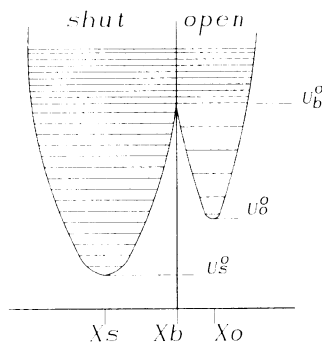

FIGURE 1 Energy level diagram of a deformable, two state channel. The ordinate is internal energy, u, and the abscissa is the reaction coordinate—distance. The energy levels are indicated diagrammatically and are not quantitatively accurate.

Two apparently contradictory models for the gating of mechanosensitive channels have been proposed. One, developed by Hudspeth and his associates (1, 2) predicts that the free energy available for gating is related *linearly* to the applied force. The other model, proposed by Guharay and Sachs (3) (see also reference 4), predicts that the free energy for gating varies with the *square* of the applied force. In this note we consider the statistical mechanics of conformational transitions between two states of an elastic channel in the presence of an external force field. The results predict both linear and quadratic terms. The linear term is larger than the quadratic term, but by how much depends upon the value of specific constants of the model.

Consider a stretch-activated (SA) channel as having two conformations, open (o) and shut (s). In each conformation, the channel protein can be deformed by an external force, F. Thus, each conformation is characterized by a Hooke's Law elasticity with a force constant K_j and an equilibrium extension x_j in the absence of applied force. Here $j = o, s$ for open and shut conformations. The potential energy of the elastic elements as a function of extension is represented by the double well model of Fig. 1. When the barrier height separating the two wells is large compared to thermal energy, $u_b \gg kT$, the two wells can be considered as independent harmonic oscillators because the fraction of time spent in the transition region, $E > u_b$, is a negligible fraction of the time spent in either of the two wells. In this case, the ratio of equilibrium probabilities for being in either conformation is just the ratio of the partition functions for each oscillator,

$$P_o/P_s = Z_o/Z_s = \exp[-(A_o - A_s)/kT], \quad (1)$$

Address correspondence to Dr. Sachs.

where Z_j is the partition function,

$$Z_j = \sum \exp(-E_{j,n}/kT),$$

with $E_{j,n}$ indicating the energy levels of conformation j; $A_j = -kT \ln Z_j$ is the Helmholtz free energy of conformation j.

Because each conformation can be regarded as a harmonic oscillator in a uniform force field, each conformation has a Hamiltonian,

$$H_j = p^2/2m + (K_j/2)(x - x_j)^2 + u_j^o - Fx,$$

where p is momentum, m is mass, u_j^o a constant zero-point potential energy, F is the applied force, and x the distance moved under that force. This Hamiltonian can be rewritten by completing the square as

$$H_j = \{p^2/2m + (K_j/2)[x - (x_j + F/K_j)]^2 + u_j^o\} - \{(Fx_j + F^2/2K_j)\}.$$

The first term in curly brackets is the Hamiltonian of the unperturbed oscillator (note the term $x_j + F/K_j$ simply represents an offset in the mean position of the oscillator which does not change the energy levels). The second term in curly brackets is the contribution of the external force.

The energy levels of H_j are

$$E_{j,n} = \{(n + \tfrac{1}{2})h\upsilon_j + u_j^o\} - \{(Fx_j + F^2/2K_j\}$$

$$= \{E_{j,n}^o\} - \{\Phi_j\}, \quad (2)$$

where $\upsilon_j = (K_j/m)^{1/2}$. We can now substitute Eq. 2 into the partition sums of Eq. 1 to get

$$Z_j = \sum \exp\{-(E_{j,n}^o + \Phi_j)/kT\} = Z_j^o \exp(\Phi_j/kT),$$

where

$$Z_j^o = \sum \exp(-E_{j,n}^o/kT)$$

$$= \sum \exp\{[-(n + \tfrac{1}{2})h\upsilon_j - u_j^o]/kT\}$$

$$= \exp(-u_j^o/kT)/[\exp(h\upsilon_j/kT) - 1].$$

Note that Z_j^o may be different for each conformation but is independent of the applied force. The Helmholtz free energies are then

$$A_j = -kT \ln Z_j^o - \Phi_j = A_j^o - \Phi_j \quad (3)$$

and the free energies of the open and shut conformations are

$$A_o = A_o^o - (Fx_o + F^2/2K_o); \qquad A_s = A_s^o - (Fx_s + F^2/2K_s).$$

These are shown as functions of applied force in Fig. 2. The free-energy difference between the two conformations is

$$\Delta A_{os} = A_o - A_s = (A_o^o - A_s^o) - F(x_o - x_s) - F^2(1/K_o - 1/K_s)$$

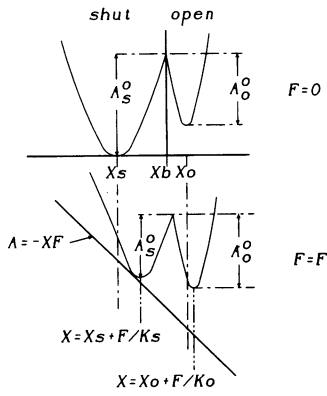$$= \Delta A_{os}^o - F\Delta x_{os} - F^2 \Delta c_{os}/2, \quad (4)$$

FIGURE 2 Free energy diagram of the channel in the absence (*upper panel*) and presence (*lower panel*) of external forces. The open state is drawn with a smaller compliance (higher stiffness). Note that when the force is applied, the barrier height, and the location of the energy minimum shifts more for the shut (more compliant) state than the open (less compliant) state.

where Δc_{os} is the difference in compliance of the closed and open states.

Substituting into Eq. 1 and noting that $p_o + p_s = 1$, gives the equilibrium probability for being in the open state as

$$p_o = [1 + \exp{(\Delta A/kT)}]^{-1}. \qquad (5)$$

For an SA channel, we would expect $A_o^o > A_s^o$, so that the channel would tend to be shut in the absence of applied force. For the model of elasticity used here, a single harmonic mode, this will be the case when the shut state is more compliant than the open state. (One might employ other models of the protein elasticity, such as the purely entropic spring. For this case, the force dependence will contain only the linear term.) For the enthalpic (harmonic mode) elasticity, Eq. 3 states

$$A_j^o = kT \ln h\nu_j/kT = kT \ln h/kT(K_j/m)^{1/2}.$$

The more compliant state, having a less steeply rising parabolic well, has more closely spaced energy levels and consequently a larger entropy,

$$S_j^o = (U_j^o - A_j^o)/T = kT\{1 + \ln{[(kT/h)(K_j/m)^{-1/2}]}\},$$

where

$$U_j^o = \sum E_{j,n}^o \exp{(-E_{j,n}^o/kT)}/Z_j^o \approx kT.$$

Referring to the lower part of Fig. 2, consider the transition barriers and the kinetics of the open–closed transitions. From the figure we see that with force applied,

$A_s = A_s^o - Fx_s - F^2/2K_s$, minimum energy of the shut state

$A_b = A_b^o - Fx_b$, energy at the activation barrier

$A_o = A_o^o - Fx_o - F^2/2K_o$, minimum energy of the open state.

The energy differences give the barriers to transition as,

$$\Delta A_{bo}^{\ddagger} = (A_b^o - A_o^o) - F(x_b - x_o) + F^2/2K_o, \qquad (6a)$$

$$\Delta A_{bs}^{\ddagger} = (A_b^o - A_s^o) - F(x_b - x_s) + F^2/2K_s. \qquad (6b)$$

It is important to estimate the relative magnitude of the linear and squared terms in Eqs. 6. The parameters cannot be varied independently since the model has been derived subject to the assumption that the time spent above the barrier is negligible, i.e., that at all forces, the energy does not become comparable to the barrier height. This constraint means that, for example, x_s cannot become equal to x_o. As a typical case, consider rewriting Eq. 6b as,

$$\Delta A_{bs}^{\ddagger} = (A_b^o - A_s^o) - F[(x_b - x_s) + F/2K_s],$$
$$= \Delta A_{bs}^o - F(\Delta x_{bs} + \Delta x_{se}).$$

The elastic strain on the shut state is $2\Delta x_{se}$. When the shut state is stressed, the energy rises toward the barrier. The available force must not increase the strain energy above the barrier otherwise we violate our assumption of independent oscillators, i.e., the barrier will disappear. Since the force must not bring the energy levels to the barrier height, $\Delta x_{se} < \Delta x_{bs}$ and the linear term is larger than the quadratic term.

In another way, the free energy difference between the open and closed states can be written as

$$\Delta A_{os} = \Delta A_{os}^o - F\Delta x_{os} + F^2\Delta c_{so},$$

where all the coefficients have been written as positive quantities ($\Delta c_{so} > 0$ when the shut channel is more compliant than the open channel). At some value of the force, $F_{1/2}$, the open and closed states are equally populated, i.e., when $\Delta A = 0$ has a root for positive F. It is easy to see that this will occur when the linear energy term is larger since

$$\Delta A_{os}^o/F_{1/2}\Delta x_{os} = 1 - F_{1/2}^2\Delta c_{so}/F_{1/2}\Delta x_{os}.$$

The left hand side is > 0 so the second term on the right must be < 1. The actual contribution of the two terms must be evaluated experimentally by quantitative analysis of the dose-response data, preferably from rate constants rather than probabilities. This data is not yet available.

If the open state is very stiff, so that $x_o \approx x_b$ and K_o is large, then $\Delta u_{bs}^{\ddagger}$ is a constant independent of F. (These two conditions are not completely independent since a stiff open channel has a narrow energy well placing it close to the barrier.) This approximation predicts an open-state lifetime distribution independent of F, the result found by Guharay and Sachs for SA channels in chick skeletal muscle (3). Note, however, that the opening rate corresponding to $\Delta u_{bs}^{\ddagger}$ depends on F as

$$k_{bs} \propto \exp{[(x_b - x_s)F/kT + F^2/2K_s kT]},$$

which has the same log slope as $p_o(F)$. The rate constants and equilibrium probabilities, however, have both linear and quadratic terms. If the two conformations had equal compliances, $K_s \approx K_o$, Eq. 4 yields the Hudspeth model.

If the channel kinetics can be measured with sufficient

accuracy, it should be possible to extract the elasticity (K_j) of each state and the distance between the energy wells and barrier (Eqs. 6). Experiments to date have not carefully measured the patch stress and strain, so the true dose-response curve is not known.

Guharay and Sachs (3) postulated that stretch-activated channels had to be attached to a tension bearing cytoskeleton because they could not otherwise account for the steepness of the dose-response relationship. Despite the fact that their theory was incomplete, the conclusion that cytoskeleton is involved seems to be correct (5). Using the linear force relationship (Eq. 4 with $K_s = K_o$), the observed sensitivity of ~1 kT/dyn/cm can be obtained with minor alterations in the channel structure, a cylindrical channel 10 nm in diameter need increase its diameter by 0.02 nm/dyn/cm. In contrast to this conclusion, Howard and Hudspeth (6) estimated from compliance studies on hair cells that the mechanosensitive channels appear to change dimensions by 4 nm. Assuming that similar channels are involved in both systems, this large difference suggests that only a small part of the channel is deformed by the applied force, i.e., the equivalent diameter of the channel might be 1 nm instead of 10 nm.

The conclusion of Howard and Hudspeth based on measurements of compliance might be affected by the results of the analysis above. Compliance is $d\langle x \rangle/dF$, where the brackets indicate mean values. Since the channel is either open or shut with probability p_o and p_s, respectively, $\langle x \rangle$ is given by

$$\langle x \rangle = \langle p_o x_o(F) + p_s x_s(F) \rangle,$$

where $x_o(F) = x_o + F/K_o$ and $x_s(F) = x_s + F/K_s$. Letting the difference in compliance of the open and shut states be $\Delta c = (1/K_o - 1/K_s)$ and taking derivatives,

$$d\langle x \rangle/dF = p_o \Delta c + (\Delta x_{os} + F\Delta c) dp_o/dF + 1/K_s. \quad (7)$$

Because $p_o(F)$ is sigmoidal, dp_o/dF is a function which peaks at the inflection point of p_o, i.e., where the channels are open half the time (in this two-state model). When p_o is low, the compliance is simply $1/K_s$ and when p_o is high the compliance is $1/K_o$. In between, the compliance changes with F. Howard and Hudspeth (6) identified the coefficient of dp_o/dF with $\Delta x_{os} = x_o - x_s$, but as seen in Eq. 7, the coefficient could be larger or smaller than Δx depending upon the sign of Δc. Using arguments as presented above, $\Delta x > F\Delta c$. If Δc_{os} is positive, Δx should not be underestimated by more than a factor of two. If Δc_{os} is negative, Δx could be significantly underestimated.

This analysis also applies to voltage sensitive channels.

Barrier models that predict an exponential dependence of the rate constants on voltage have implicitly made the assumption that the channels do not distort in the applied field until they suddenly change state. This assumption cannot be precisely obeyed, so that the voltage dependence of rate constants should involve second order (or higher) terms in voltage, as well as the usual linear term and the higher order terms would become more important at the higher field strengths.

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Frederick Sachs* and Harold Lecar‡

*Department of Biophysical Sciences,
State University of New York,
Buffalo, New York 14214; and
‡Department of Molecular and Cell Biology,
Division of Biophysics,
University of California,
Berkeley, California 94720 USA